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Competitive Formation of *N*-Diazeniumdiolates and *N*-Nitrosamines via Anaerobic Reactions of Polyamines with Nitric Oxide

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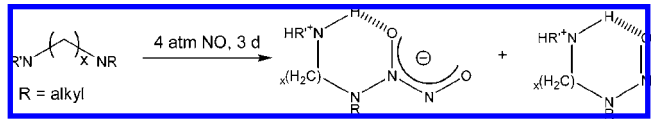
ABSTRACT



Reactions of amines with nitric oxide (NO) at high pressures form diverse NO donor species, highly dependent on the precursor structure. While monoamine precursors favor the formation of *N*-diazeniumdiolates in high yield, polyamines exhibit competitive formation of *N*-nitrosamines and diazeniumdiolates, resulting in mixed products containing significant percentages of undesired *N*-nitroso compounds.

The role of nitric oxide (NO) in physiological signaling pathways, vasodilation, wound healing, and platelet aggregation has stimulated much research related to the molecular storage of NO.[1−5] Formulations of scaffolds modified with functionalities capable of storing and releasing NO have been developed as both tools for further elucidating NO's role in physiology and as potential therapeutics.[6−12] Among the numerous types of NO-donating compounds, *N*-diazeniumdiolates are among the most widely researched donors due to their straightforward synthesis, long-term storage capabilities, and ability to spontaneously release NO under physiological conditions.[6,8]

Although the first report regarding the synthesis of *N*-diazeniumdiolates occurred almost 50 years ago,[13,14] it was not until the identification of NO as a ubiquitous signaling molecule in the early 1990s that synthetic strategies to manipulate NO storage and release, and associated pharmacological effects were significantly accelerated.[6,15] Two primary hypotheses regarding the mechanism of diazeniumdiolate formation under anaerobic conditions have been proposed differing only in the reactive state of NO. In the first, NO reacts with an amine to form the nitrosamine radical anion that subsequently reacts with another molecule of NO to form the diazeniumdiolate.[16−18] In contrast, others have proposed that NO first dimerizes to N_2O_2 and then acts electrophilically in coupling with the amine to form the

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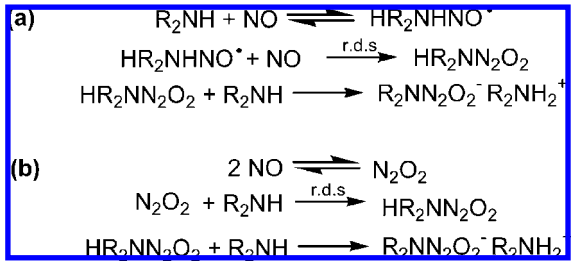
diazeniumdiolate.[16] Using the mechanistic understanding from the hypothesis of sequential NO addition, herein we demonstrate experimentally that nitrosamines form competitively with diazeniumdiolates in anaerobic reactions of NO with polyamines.

Previous work with secondary amines indicates efficient diazeniumdiolate formation in situ under high pressures (4−5 atm) of NO and basic conditions.[19−21] According to the mechanisms first proposed by Drago and investigated by others,[16−18] the rate limiting step of diazeniumdiolate formation is the second addition of NO to the intermediate nitrosamine radical anion. As such, low NO concentrations and/or slow rate determining steps (Scheme 1) may facilitate

Scheme 1. Mechanisms of Diazeniumdiolate Formation: (a) Sequential NO Addition, (b) Dimer Addition[16−18]



incomplete diazeniumdiolate formation from nitrosamine intermediates, which despite their ability to controllably release NO in the presence of light, are widely considered to be carcinogenic.[7]

To assess the possibility of nitrosamine formation, a number of monoamine and polyamine species (Figure 1)

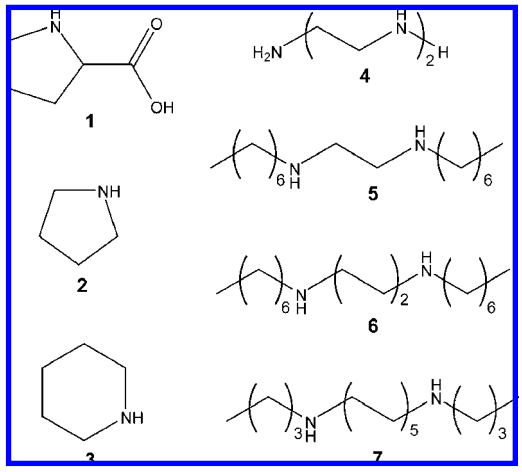


Figure 1. Amine compounds investigated: (**1**) proline, (**2**) pyrrolidine, (**3**) piperidine, (**4**) diethylenetriamine (DETA), (**5**) *N*,*N*′-diheptyl ethylenediamine (DHED), (**6**) *N*,*N*′-diheptyl-1,4-butylenediamine (DHBD), (**7**) *N*,*N*′-dibutyl decylenediamine (DBDD).

were synthesized and treated with high pressures (4 atm) of NO for 3 d. Care was taken to remove oxygen from the reaction vessel in order to minimize nitrosamine formation via alternative pathways mediated by NO_2.[22] Nitric oxide release from the products was quantified under either standard physiological conditions (pH 7.4 phosphate buffered saline, 37 °C) or highly basic conditions (1.0 M NaOH) in the presence of direct 200 W broad spectrum light. The degradation of *N*-diazeniumdiolates to NO and the parent amine in phosphate buffered saline,[6,15] and the stability of nitrosamines in aqueous solutions at physiological pH[7] are well-known. Similarly, direct light irradiation in basic solutions was chosen for the detection of nitrosamine-derived NO due to the known photodegradation of nitrosamines and stability of diazeniumdiolates under basic conditions.[7,15]

Unexpectedly, more pronounced NO release was observed with light irradiation compared to physiological conditions for diethylenetriamine (**4**) and *N*,*N*′-diheptyl ethylenediamine (**5**) (Figure 2), indicating a mixture of products, including

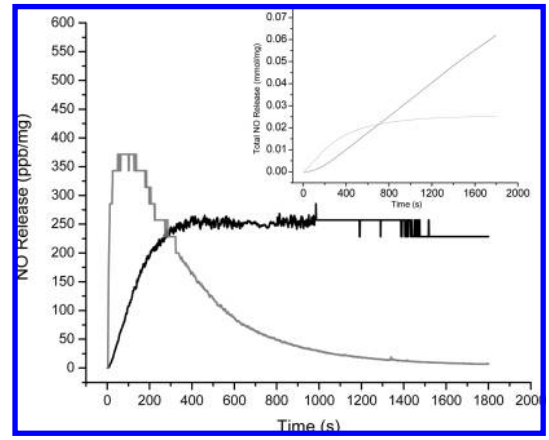


Figure 2. Real-time and total (inset) NO release of NO-treated **5** analyzed in the presence of 1.0 M NaOH, light (black), and PBS, pH 7.4 (gray).

both the *N*-nitroso and the *N*-diazeniumdiolated species. When the monoamine compounds proline (**1**), pyrrolidine (**2**), and piperidine (**3**) were exposed to similar conditions, NO release in the presence of light was minimal, indicating preferential formation of *N*-diazeniumdiolates over nitrosamines (Table 1).

Absorption spectroscopy was used to confirm the formation of both *N*-nitroso and *N*-diazeniumdiolate products on polyamine precursors (**4**, **5**, *N*,*N*′-diheptyl- 1,4-butylenedi-

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Table 1. NO Release Characteristics of Monoamine and Polyamine Compounds Exposed to 4 atm NO for 72 h

amine precursor	NO initiation method	NO release (μmol/mg)[a]	nitrosamine/ diazeniumdiolate[b]
1	Light	0.04 ± 0.01	1.00/49.75
	Proton	3.98 ± 0.49	
2	Light	0.06 ± 0.01	1.00/97.09
	Proton	11.67 ± 0.65	
3	Light	0.06 ± 0.01	1.00/47.85
	Proton	5.74 ± 0.22	
4	Light	1.14 ± 0.16	1.00/0.38
	Proton	0.86 ± 0.09	
5	Light	2.16 ± 0.17	1.00/0.01
	Proton	0.04 ± 0.01	
6	Light	1.58 ± 0.44	1.00/1.44
	Proton	4.56 ± 0.51	
7	Light	1.47 ± 0.08	1.00/1.43
	Proton	4.21 ± 0.31	

[a] Total after 32 min analysis for monoamine compounds and 16 h analysis for polyamine compounds based on duration of NO release. [b] Calculated from total NO release assuming one and two molecules of NO for nitrosamine and diazeniumdiolates respectively.

amine (**6**), and *N*,*N*′-dibutyl-1,10-decylenediamine (**7**)). Absorbance at 252 nm, indicative of *N*-diazeniumdiolates,[6] was most intense for monoamine compounds **1**−**3**, but visible for all species treated with NO except for **5**, which exhibited almost 100-fold greater preference for nitrosamine formation (Table 1). All NO-treated polyamine compounds showed supplementary absorbance values between 330−350 nm associated with the n→π* transition of *N*-nitroso compounds (Figure 3).[7] Of note, the molar absorptivities of these two

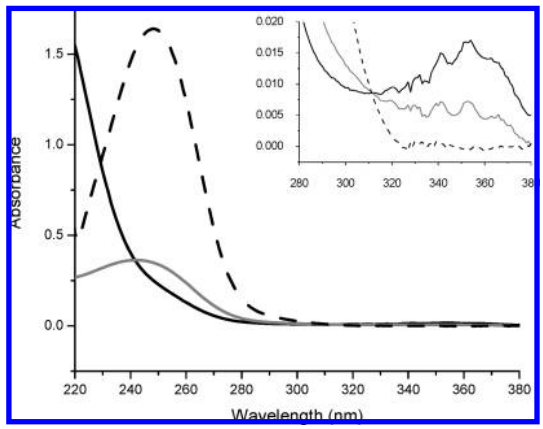

Figure 3. UV/vis spectra for NO treated **2** (dashed black), **5** (solid black), and **6** (solid gray) in MeOH. [Inset: Expanded view of *N*-nitrosamine absorbance region.]

functional groups spanned several orders of magnitude, from 40−90 M^{-1} cm^{-1} for the nitrosamine peaks of the synthesized polyamines to 7.2−9.4 × 10^3 M^{-1} cm^{-1} for diazeniumdiolates.[6]

The preference for diazeniumdiolate versus nitrosamine formation seems to be influenced by the structure of the amine precursor. Intramolecular hydrogen bonding is possible on the nitrosamine radical anion intermediate formed during diazeniumdiolate formation for polyamine compounds, but not monoamines. Specifically, neighboring amines may act to stabilize the nitrosamine intermediate in compounds derived from **4** and **5** in the form of N−H···O═N, which has recently been shown to be more stable than N−H···N═O.[23] The lack of multiple amines on other compounds such as **1**−**3** do not allow for this type of stabilization. While fully formed diazeniumdiolates and nitrosamines may both experience similar hydrogen bond stabilization, interruption of the reaction progression at the intermediate stage due to hydrogen bonding impedes the sequential addition of NO and thus the complete conversion to the diazeniumdiolate species. Although the influence of hydrogen bonding is decreased in polar solvents, collapse of the hydrophobic alkyl segments of the molecules may further facilitate such stabilizing interactions due to proximity of amines after chain collapse. Representative NO release curves for the monoamine **1** after treatment with NO indicate a strong preference of diazeniumdiolation, as NO release due to light is insignificant compared to that occurring in buffer immersion alone (Figure 4).

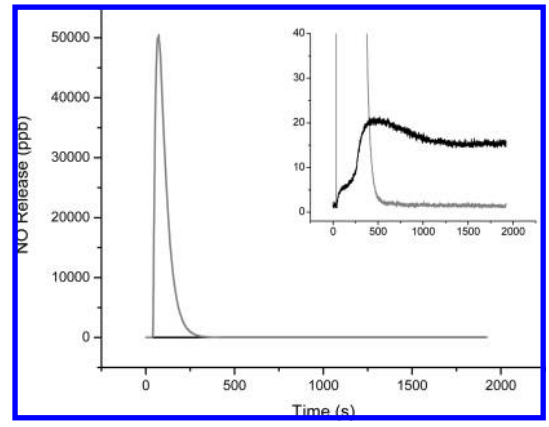

Figure 4. Real-time NO release of NO-treated **1** analyzed in 1.0 M NaOH with direct light (black), and PBS pH 7.4 (gray). [Inset: enlarged view of NO flux in 1.0 M NaOH with direct light.]

Additional evidence supporting the hydrogen-bonding dependence on the observed nitrosamine products is confirmed with other polyamine compounds. As the amine spacing of polyamine compounds was increased from ethylene for **5** to butylene for **6** (as well as ethylene (**5**) to decylene (**7**)) the calculated nitrosamine:diazeniumdiolate ratio decreased (NO-release analysis, Table 1). Additionally, the UV absorbance due to nitrosamines decreased for **6** and **7** relative to **5**, with a concomitant increase in the diazeniumdiolate absorbance (Figure 3). The shift from nitrosamine to diazeniumdiolate formation may be attributed to the

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increased strain associated with the formation of larger ring systems necessary for the intramolecular hydrogen bond stabilitization on the nitrosamine intermediate. The decreased ability for nitrosamine stabilization may facilitate the second NO addition, thus increasing the efficiency of diazeniumdiolate formation.

To address the incomplete diazeniumdiolate formation, alterations of the NO addition reactions were made. Experiments were performed by increasing both the total reaction time of the NO addition from 72 to 120 h and the amount of NO available for reaction by increasing the pressure of the NO reactor from 4 to 10 atm (maintaining a reaction time of 72 h). In both instances, the presence of nitrosamines was still observed for the polyamine compounds of interest (data not shown).

Spectroscopic and NO-release analysis indicates that both diazeniumdiolates and nitrosamines form competitively with exposure of polyamines to high pressures of NO. The lack of stabilizing intramolecular hydrogen bonds for monamine compounds (e.g., **1**−**3**) facilitates the conversion of nitrosamine intermediates to the intended diazeniumdiolates. However, molecules capable of forming intramolecular hydrogen bonds display an impeded level of diazeniumdiolate formation due to the increased stability of the nitrosamine intermediate. As the effects of NO derived from diazeniumdiolate degradation has been reported to be beneficial by numerous researchers, the results presented here indicate that the increased incidence of nitrosamine formation on polyamine scaffolds may pose concern for some applications of these NO donors. In contrast, the suitability of monoamine species as diazeniumdiolate donors is supported by minimal nitrosamine formation. Overall, the results suggest that current synthetic methods may require revision to minimize nitrosamine formation on polyamine compounds, or at least stringent characterization to confirm their absence.

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Supporting Information Available: Synthetic and experimental procedures and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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